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Suppression of cogongrass (*Imperata cylindrica*) by a bioherbicidal fungus and plant competition

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The possibility of using the fungus *Bipolaris sacchari* as a bioherbicide to suppress cogongrass and to allow the establishment of bahiagrass in cogongrass–bahiagrass mixed plantings was investigated under greenhouse conditions. The bioherbicide was prepared by mixing *B. sacchari* spore suspension containing 10^5 spores ml^{-1} with an oil emulsion composed of 16% horticultural oil plus 10% light mineral oil and 74% sterile water. The bioherbicide caused severe foliar blight in cogongrass and slight phytotoxic damage on bahiagrass. In the first experiment, the bioherbicide reduced cogongrass biomass without affecting bahiagrass biomass. In the second experiment, the bioherbicide caused a 64% reduction in fresh weight, a 74% reduction in the number of rhizomes, and a 47% reduction in the height of cogongrass. The latter experiment also showed an increase in bahiagrass fresh weight in the presence of cogongrass when the bioherbicide was applied. This study indicates the potential of combining bioherbicide application with competition from a desirable grass species as a strategy for the integrated management of cogongrass.

Nomenclature: Bahiagrass, *Paspalum notatum* Fluegge var. *saurae* Parodi PASNO 'Pensacola'; *Bipolaris sacchari* (E. J. Butler) Shoemaker; cogongrass, *Imperata cylindrica* (L.) Beauv. IMPCY.

Key words: Biological control, bioherbicide, competition.

Cogongrass has been ranked as the seventh worst weed in the world and the most serious perennial weed of southern and eastern Asia (Holm et al. 1977). It infests over 500 million ha worldwide, including 200 million ha in Asia and several thousand hectares in the southeastern United States (Dickens 1974; Falvey 1981; Holm et al. 1977). It is a weed of 35 economically important crops in 73 tropical countries (Holm et al. 1977). In the United States, cogongrass is not a weed in most cropping areas, but it is regarded as one of the most invasive plant species in natural and disturbed areas (Coile and Shilling 1993). It occurs in Florida, Georgia, Alabama, Mississippi, Louisiana, South Carolina, and Texas (Van Loan et al. 2002).

Cogongrass spreads frequently over large areas and excludes other grasses by forming dense mats of branched rhizomes and by releasing allelochemicals. It can hamper the establishment of desirable plant species by extracting moisture and nutrients from the shallow layers of soil. It is able to invade areas that will not support the growth of other grasses because it can tolerate a wide range of soil conditions (Hubbard et al. 1944). Cogongrass is capable of reinvading an ecological niche that is not filled with another plant species after control methods have been implemented. Therefore, effective and long-term control should consist of methods for cogongrass suppression followed by the establishment of desirable plant species that will replace cogongrass (Shilling et al. 1997).

Gaffney (1996) evaluated the effect of using chemical herbicides and two plant species, bermudagrass [*Cynodon dactylon* (L.) Pers.] and hairy indigo (*Indigofera hirsuta* Harvey), in suppressing cogongrass and preventing its reinfestation. He observed that the application of herbicides and the presence of the two plant species suppressed cogongrass for up to 2 yr after seeding. According to Gaffney, the success of this strategy relies on the tolerance of the desirable species to the herbicides used (selective control) and conditions favorable for their growth.

Among the plant species that are infested by or are at risk of being infested and displaced by cogongrass is bahiagrass (Shilling et al. 1997; Willard and Shilling 1990). At present, bahiagrass is the most common turf species used for soil stabilization and beautification on Florida rights-of-way (Beard 1980). It is extensively planted along the highways of Florida and in other subtropical and mild temperate areas because of its ability to thrive in warm weather and in dry and infertile soils with low pH. Bahiagrass is also ideal for turf because it requires little or no irrigation and minimal fertilization and has relatively few pest problems (Anonymous 2000). However, it grows poorly under moderate or heavy shade. Because of their slow growth rate, bahiagrass seedlings are weak competitors and are susceptible to competition from aggressive grass species such as cogongrass (Busey and Myers 1979; Watson and Burson 1985).

Yandoc (2001) discovered an isolate of *Bipolaris sacchari* that can cause leaf lesions and severe foliar blighting on cogongrass. In greenhouse trials, it was determined that formulating the spores of this fungus with an oil emulsion (composed of horticultural oil and light mineral oil) resulted in 100% cogongrass mortality. In field trials, *B. sacchari* spores applied with 26% oil emulsion caused $>$ 70% foliar blighting. Initial host range tests indicated that the application of *B. sacchari* spores formulated in oil emulsion did not severely damage bahiagrass.

The objective of this study was to determine whether a bioherbicidal formulation of *B. sacchari* could be used to selectively suppress cogongrass and allow for the dominant establishment of bahiagrass.

Materials and Methods

Plant Material

Cogongrass plants were propagated from rhizomes collected from a natural cogongrass stand near Lake Alice on the University of Florida campus, Gainesville, FL. Bahiagrass plants were grown from seed.[1] For all experiments, mature cogongrass and bahiagrass plants were trimmed and weighed before they were transplanted into plastic pots (27 cm diameter, 23 cm height) containing a commercial potting medium.[2] Test plants were watered regularly and fertilized as required with 10 g of Multicote® 15:15:15 N–P–K fertilizer.[3]

All plants were kept in a greenhouse that had an average day/night temperature of 35/25 ± 5 C. At midday, the relative humidity (RH) in the greenhouse was around 85 ± 5%, and natural light intensity was 400 μmol m^{-2} s^{-1}.

Fungal Inoculum

Spores (inoculum) were produced by culturing *B. sacchari* on autoclaved rye (*Secale cereale* L.) grain for 12 to 14 d at room temperature. The inoculated grains were rinsed with sterile water to recover the spores. The spores were then collected by filtering the spore suspension through a rectangular piece (42 by 48 cm) of 10-μm nylon screen.[4] The spores on the nylon screen were dried inside a fume hood without airflow for 24 to 48 h. The four corners of the nylon screens were held together with paper clips and fastened to a string located 30 cm below a lighted 0.9-kW incandescent bulb. The average temperature inside the fume hood was 23 C, and the RH was 55%. The dry spores were transferred to sterile glass vials and stored at 10 C until use.

The oil emulsion formulation was used in all the experiments because the addition of oil emulsion had been found to increase the biocontrol efficacy of the fungus in earlier studies. The spore and oil emulsion mixture (referred to herein as "bioherbicide") was prepared by resuspending dry spores of *B. sacchari* in sterile water and adding Sunspray® 6E horticultural oil[5] and light mineral oil[6] in a 74:16:10 v/v/v proportion (referred to as the "26% oil emulsion"). All spore and oil emulsion mixtures used in the experiments contained 10^5 spores ml^{-1}. The viability of the rehydrated spores was tested by plating the spore suspension on water agar and determining the percentage of germination after incubation at 25 C for 2 h. The viability of spores used in the experiments ranged from 94 to 99%.

Effect of Plant Competition with and without Bioherbicide Application on Cogongrass and Bahiagrass

Experiment 1

This experiment was conducted to evaluate the effect of (1) no competition (NC; cogongrass alone or bahiagrass alone), (2) plant competition (PC; cogongrass plus bahiagrass), and (3) plant competition plus bioherbicide (PC + B; cogongrass plus bahiagrass plus bioherbicide) on cogongrass and bahiagrass growth. The experiment was a completely randomized design with three replications per treatment; each replicate consisted of six plants of the same species (without competition) or three plants of each grass species (with competition). Cogongrass and bahiagrass plants were grown from planting materials that had comparable weight. The experiment was performed twice.

For the plant competition plus bioherbicide treatment (PC + B), cogongrass–bahiagrass mixtures were inoculated with the bioherbicide at 5 wk after transplanting. Noninoculated cogongrass–bahiagrass mixtures were used as controls. Treatment with oil emulsion alone as another control was omitted because it had been previously shown that the damage on cogongrass resulted from the combined effects of the fungus and the oil emulsion and not from the emulsion alone.

All inoculations were done in the greenhouse under the conditions described above. To ensure disease development, the bahiagrass–cogongrass mixtures were reinoculated at 5 d after the first inoculation. The mixtures were sprayed with the bioherbicide until runoff. The effect of plant competition alone (PC) and plant competition plus bioherbicide (PC + B) on bahiagrass and cogongrass growth was measured at 12 wk after the second inoculation by determining the combined belowground and aboveground fresh weight (g $plant^{-1}$).

Experiment 2

A second experiment was done to determine the effect of (1) no plant competition and no bioherbicide (NC; cogongrass only), (2) bioherbicide only (NC + B; cogongrass plus bioherbicide), (3) plant competition only (PC; cogongrass plus bahiagrass), and (4) plant competition plus bioherbicide (PC + B; cogongrass plus bahiagrass plus bioherbicide) on cogongrass weight, rhizome production, and plant height. The experiment was a completely randomized design with three replications per treatment. Cogongrass monocultures and cogongrass–bahiagrass mixtures were inoculated with the bioherbicide at 5 wk after transplanting. To ensure disease development, the plants were inoculated twice inside the greenhouse (first trial) or incubated in the dew chamber (27 C, 100% RH) for 7 h right after inoculation and then returned to the greenhouse (second trial). The test plants were observed weekly from the first week after inoculation until the fifth week after inoculation. At 5 wk after inoculation (WAI), cogongrass fresh weight (aboveground and belowground), plant height, and number of rhizomes produced by each plant were determined. Bahiagrass fresh weights in inoculated (PC + B) and noninoculated (PC) cogongrass–bahiagrass mixtures also were determined.

Data Analysis

All cogongrass and bahiagrass fresh weight, rhizome production, and plant height data were subjected to analysis of variance. Data from two trials of each experiment were pooled when variances were homogenous; otherwise, data from each trial were analyzed separately. The means were separated using Duncan's multiple-range test at the 5% level.

Results and Discussion

Experiment 1

The first inoculation did not produce any disease symptoms or phytotoxic damage in either plant species after 3 d.

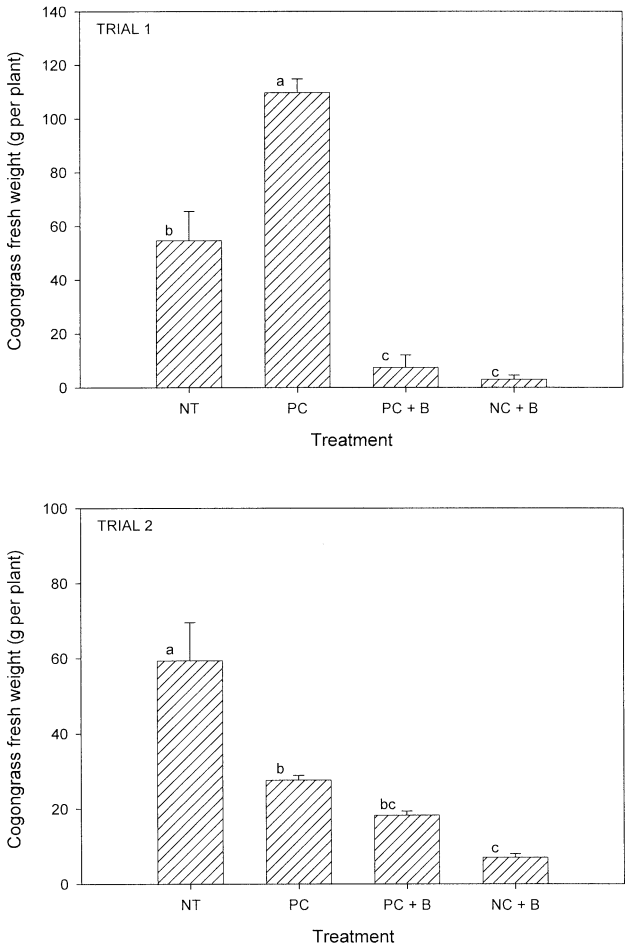

FIGURE 1. Fresh weight of cogongrass as affected by no plant competition and no bioherbicide application (NT), competition from bahiagrass (PC), competition from bahiagrass plus bioherbicide application (PC + B), and bioherbicide application alone without plant competition (NC + B). Bars are means of three replicates (from one trial); vertical lines indicate standard error. Bars with the same letters are not significantly different at P = 0.05, as determined by Duncan's multiple-range test.

Cogongrass exhibited foliar lesions only at 3 to 4 d after the inoculation was repeated. Inoculated cogongrass plants were severely blighted at 7 d after the second inoculation, whereas bahiagrass plants showed some phytotoxic damage. In both trials, the inoculated cogongrass plants were either severely blighted or dead at 12 wk after the second inoculation. Cogongrass did not regrow in the first trial. However, in the second trial, a few new cogongrass plants emerged from the treated mixtures, indicating that some of the rhizomes were still able to produce new shoots.

Cogongrass fresh weight data revealed a significant treatment effect ($P < 0.0001$). Cogongrass fresh weight from the PC + B treatment was the lowest (8 g plant^{-1}), whereas the noninoculated cogongrass planted with bahiagrass (PC) had the highest fresh weight (87 g plant^{-1}). Cogongrass planted in monoculture had an average weight of 49 g plant^{-1}.

Bahiagrass exhibited some visual injury from the bioherbicide, and its growth ranged from sparse to dense when it was coplanted with cogongrass. Bahiagrass in monoculture had significantly higher fresh weight (66 g plant^{-1}) than in the other two treatments. Bahiagrass fresh weights when planted with cogongrass (39 g plant^{-1}) and when planted with cogongrass and inoculated with the bioherbicide (17 g plant^{-1}) were not significantly different.

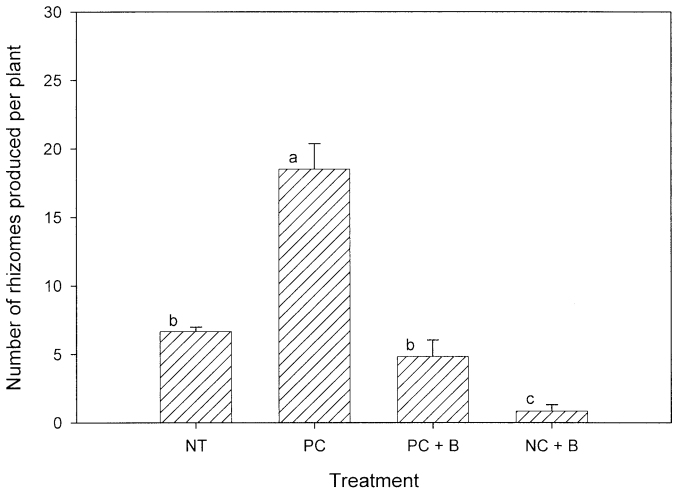

FIGURE 2. Number of rhizomes produced per cogongrass plant as affected by no plant competition and no bioherbicide application (NT), competition from bahiagrass (PC), competition from bahiagrass plus bioherbicide application (PC + B), and bioherbicide application alone without plant competition (NC + B). Bars are means of six replicates (from two trials); vertical lines indicate standard error. Bars with the same letters are not significantly different at P = 0.05, as determined by Duncan's multiple-range test.

Experiment 2

At 2 WAI, distinct foliar blighting was observed in inoculated cogongrass in monocultures or in mixed culture with bahiagrass. By 3 WAI, most of the inoculated cogongrass plants were either dead or severely blighted. Although severely blighted plants did not show signs of recovery until 3 WAI, the rhizomes produced green and healthy leaves to a small extent between 3 and 5 WAI. Inoculated bahiagrass exhibited only minor phytotoxic damage on a few leaf tips, and the plants fully recovered.

Analysis of data indicated that treatment had a significant effect on cogongrass fresh weight ($P < 0.0001$ for trial 1; $P = 0.0006$ for trial 2), number of rhizomes produced ($P < 0.0001$), and plant height ($P < 0.0001$). The bioherbicide applied to cogongrass in monoculture (NC + B) or in a mixture with bahiagrass (PC + B) significantly reduced fresh weight, number of rhizomes produced, and plant height of cogongrass. Untreated cogongrass in monoculture (NC) or in mixed planting with bahiagrass (PC) had greater weight, number of rhizomes, and plant height than cogongrass in monoculture treated with the bioherbicide (NC + B) or in mixed culture with bahiagrass when inoculated with the bioherbicide (PC + B) (Figures 1–3).

Bahiagrass fresh weight data were analyzed separately for each trial because of heterogeneous variance between trials. However, there was a consistent treatment effect on bahiagrass fresh weight ($P = 0.0018$ for trial 1; $P = 0.0108$ for trial 2). Bahiagrass planted with cogongrass and treated with the bioherbicide (PC + B) had greater fresh weight than the untreated bahiagrass planted with cogongrass (PC) (Figure 4).

The use of nonselective chemical herbicides to manage cogongrass is difficult in situations where desirable species

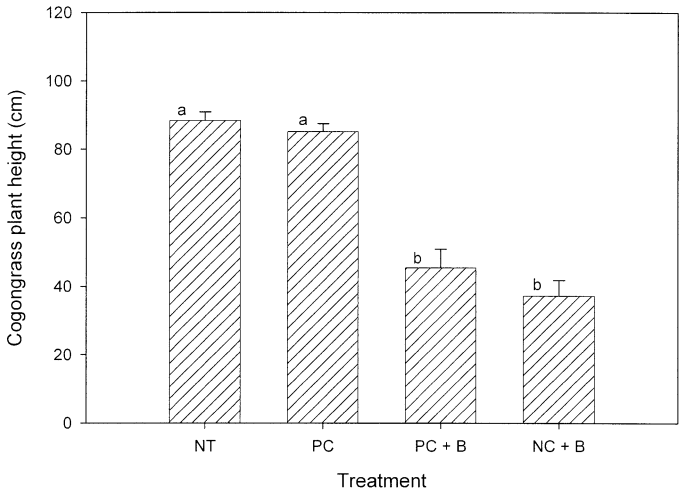

FIGURE 3. Plant height of cogongrass as affected by no plant competition and no bioherbicide application (NT), competition from bahiagrass (PC), competition from bahiagrass plus bioherbicide application (PC + B), and bioherbicide application alone without plant competition (NC + B). Bars are means of six replicates (from two trials); vertical lines indicate standard error. Bars with the same letters are not significantly different at P = 0.05, as determined by Duncan's multiple-range test.

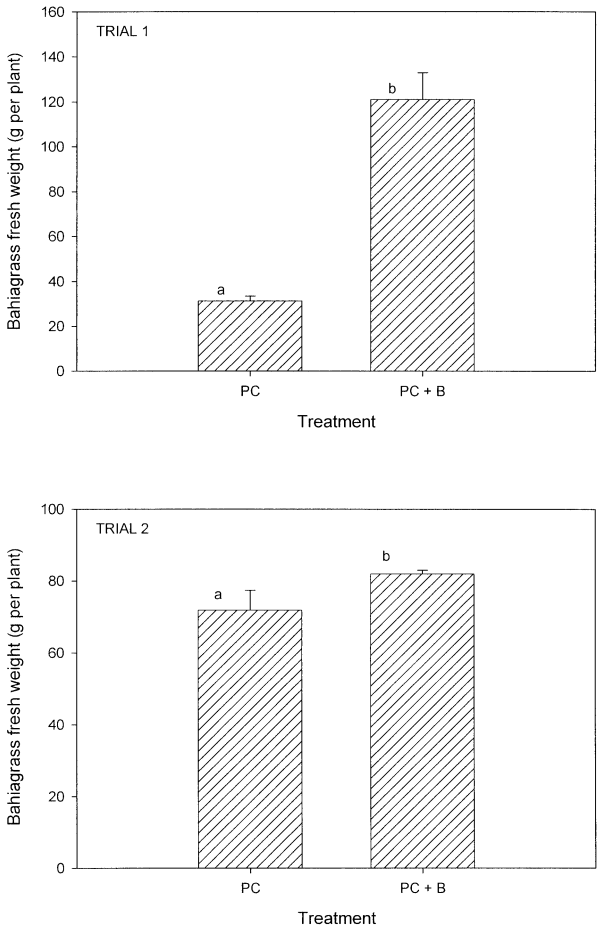

FIGURE 4. Fresh weight of bahiagrass as affected by cogongrass alone (PC) and cogongrass plus bioherbicide application (PC + B). Bars are means of three replicates (from one trial); vertical lines indicate standard error. Bars with the same letters are not significantly different at P = 0.05, as determined by Duncan's multiple-range test.

are present. Hence, systems where the desirable species and the target weed are closely related, as in the case of grasses, are examples where host-specific fungal pathogens can be exploited as bioherbicides (Templeton et al. 1979). The results from this study indicate that the damage from the bioherbicide application can substantially reduce cogongrass fresh weight, plant height, and number of rhizomes produced in monoculture and in cogongrass–bahiagrass mixtures and that bahiagrass growth in the presence of cogongrass could be enhanced when the bioherbicide is applied.

The inability of biological control agents to eliminate weeds or provide control levels similar to those of chemical herbicides has been a deterrent in the commercialization of many biocontrol agents (Rosskopf et al. 1999). However, biological control agents can provide effective weed suppression without completely eliminating the target weed (Paul and Ayres 1986; Watson and Wymore 1990). There are situations, as demonstrated here, where biocontrol agents can be used in conjunction with other existing weed control measures to effectively suppress a target weed. Because pathogens can negatively affect plant growth and reproduction (Clay et al. 1989), they can be used to cause additional stress to weeds (Groves and Williams 1975; Paul 1989) and reduce their ability to effectively compete with other plant species that occupy the same niche.

The mediating effect of host-specific pathogens on intra- and interspecific competitive interactions has been demonstrated by several previous studies (Ayres and Paul 1990; Burdon et al. 1984; Paul and Ayres 1986). According to Bruckart and Hasan (1991), the use of a beneficial competitor along with the biological control agent may serve three purposes: (1) to increase the rate of weed control, (2) to fill the niche vacated with a plant species of value, and (3) to reduce the probability that the weed will become permanently established. This study, although conducted only in a greenhouse, has demonstrated the potential value of *B. sacchari* for integrated management of cogongrass. Additional field experiments are needed to validate the feasibility of using the bioherbicide to suppress cogongrass growth and enable a desirable species, such as bahiagrass, to become established in natural areas.

Sources of Materials

[1] Bahiagrass seeds were obtained from Dr. G. Miller of the Department of Environmental Horticulture, University of Florida, Gainesville, FL 32611.

[2] MetroMix® 300, Scott-Sierra Horticultural Products Co., 14111 Scottslawn Road, Marysville, OH 43041.

[3] Multicote®, Scott-Sierra Horticultural Products Co., 14111 Scottslawn Road, Marysville, OH 43041.

[4] Nytex screen, Sefar America Inc., 111 Calumet Street, Depew, NY 14043.

[5] Sunspray® 6E, Sun Company Inc., P.O. Box 1135, Marcus Hook, PA 19061-0835.

[6] Light mineral oil, Fisher Scientific, 1 Reagent Lane, Fair Lawn, NJ 07410.

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